UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

March 22, 2008

MEMORANDUM

Subject: Efficacy Review for EPA File Symbol No. 74234-R, LMP-102;
DP Barcode: 346435

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch 5/22/08
Antimicrobials Division (7510P)

Thru: Michele Wingfield, Chief
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To: Velma Noble PM 31/ Jacqueline Campbell
Regulatory Management Branch I
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Applicant: Intralytix, Inc.
3232 W. Camden Street
Baltimore, MD

Formulations from Label

Active Ingredient(s)	% by wt.
Listeria Specific Bacteriophage	0.00001%
Other Ingredients	99.9999%
Total	100.00000%

I BACKGROUND

The product, LMP-102™ is a lytic phage cocktail against *Listeria monocytogenes.* LMP-102™ is for use in the control of *L. monocytogenes* on non-food contact surfaces in food processing (poultry, meat, pork, etc.) and food handling establishments. Per the label, use LMP-102™ as part of an integrated microbial control program with EPA approved sanitizers. The submitted efficacy study was conducted at MicroBioTest, Inc., located at 105B Carpenter Drive, Sterling, VA, 20164.

The data package contained a letter from the applicant's representative to the EPA (dated September 23, 2007), Statement of No Data Confidentiality, GLP Compliance Statement, one efficacy study (MRID No. 472420-01), and the proposed label.

II USE DIRECTIONS

LMP-102 (Non-Food Contact Sanitizer) as part of an integrated microbial control program with an EPA approves sanitizer can be used to treat walls, floors, drains, grating, and other non-food contact equipment. Directions on the proposed label provided the following instructions for the preparation and use of the product as a non-food contact sanitizer:

Apply LMP-102 prior to or after the application of the sanitizer. Apply the product to surfaces by spraying or with a cloth or sponge. Apply sufficient amounts of LMP-102 so that the target surface is thoroughly covered. About 50 ml of LMP-102 will treat approximately 4½ ft2 surface. Allow LMP-102 to remain on the treated surface for a minimum of 5 minutes.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Sanitizer (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. Testing requirements in EPA DIS/TSS-10 may be used. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. These Agency standards are presented in DIS/TSS-10.

IV SYNOPSIS OF SUBMITTED EFFICACY STUDY

1. MRID No. 472420-01, "Sanitizer Test for Non-Food Contact Surfaces" for Test Agent LMP-102" by Felicia Sellers. Study completion date—September 10, 2007. Laboratory Project Identification Number—581-102.

This study was conducted against *Listeria monocytogenes* (ATCC 35152-serogroup 1/2a, 13932-serogroup 4b, and Lm 301-serogroup 1/2a). The test culture was prepared by pooling equal volumes (5 ml) of each strain. Three lots (Lot Nos. 0107F040117, 0107H150101, and 0107H150202) of the product, LMP-102, were tested. The laboratory study referenced the Sanitizer Test from DIS/TSS-10 (test protocol included). At least one of the product lots tested (i.e., Lot No. 0107F040117) was at least 60 days old at the time of testing. The organic soil load (5%) as achieved by adding 10 µl of 5% skim milk (dried) on the surface of each carrier prior to "overlayment" with the test culture inocula.

Test Article Preparation:
LMP-102 was stored refrigeration (2-6°C) in a dark place. Test substance was recived ready-to-use. The potency/titer was of the test substance was verified before shipping. Potency/titer of LMP-102 should be $1 \times 10^9 \pm 0.5$ log PFU/ml.

Inoculum Preparation:
--Each of the test *L. monocytogenes* strains from the stock culture were streaked onto separate MOX agar plates, and incubated at 30±2°C for 18±2 hours.
Note: It is acceptable to use a single colony from a previously inoculated MOX plate stored at 2-8°C for up to two weeks.

--A single colony of each strain is subcultured into 10 ml of LB broth and incubated at 30±2°C with shaking (at approximately 150 rpm) for 18±2 hours. The identity of each of the three strains was confirmed.

--Each of the three bacterial cultures were separately passed through a sterile funnel containing cheesecloth (one layer of ca. 4 ply) to remove any particulates.

--The OD_{600} for each of the three strains was determined and recorded.

--The concentration of each test strain was adjusted to 5×10^6 -1×10^7 to the final volume of 10 ml. In most cases, this was achieved by diluting 100 µl of culture in 9.9 ml of LB broth.

--Equal volumes (5 ml each of all 3 diluted test strains were mixed together.
Note: The three *L. monocytogenes* strains were mixed in essentially equal concentrations in the "Test Culture", and the numbers of bacteria recovered from the PBS treatment group should be within the following range: $5 \times 10^3 - 2 \times 10^4$ CFU/coverslip.

--Combine test cultures were used within 60 minutes of mixing.

Carrier Preparation:

--Three cover slips were prepared for (i) each lot of test agent to be tested, (ii) carrier (PBS) treatment group, and (iii) zero time bacterial recovery group. Six cover slips were prepared for the neutralizer group.

--To simulate an organically soiled surface, 10µl of a 5% skim milk solution was transferred onto the center of each carrier and spread uniformly to an area approximately 4-6 mm in diameter. Ten (10) µl of the test culture mixture was applied directly over the skim milk spot on the surface of each cover slip.

--Contaminated carriers were allowed to dry at room temperature (actual temperature and drying time not included).

Test:

--100 µl of LMP-102 was transferred onto the surface of contaminated test carriers.

--The test agent was allowed to remain in contact with the inoculated test surface for 4.5 minutes at 23-27°C.

--At the completion of the contact time, the carriers were immediately removed from the Petri dish with sterile forceps and held above the filter paper to drain off any excess product.

--The carriers were transferred into a 50 ml conical tube containing 20 ml of sterile peptone water and mixed vigorously for approximately 30 seconds.

--Ten-fold serial dilutions were immediately performed through 10^{-2} in peptone water (20 ml final volume).

--This test mixture 10^{-2} (20 ml) was filtered through a 0.45 µM Nalgene filter.

--The filter was washed with 20 ml of sterile peptone water and then placed (upside down) on a MOX agar plate and incubated at 30±2°C for 24-26 hours.

-- Colonies were counted and CFU/cover slip calculated.

Controls included zero time bacterial numbers recovery group and sterility controls.

V RESULTS

Challenge Strain	Average CFU/ml
Listeria monocytogenes (ATCC 35152)	6.5×10^6
Listeria monocytogenes (ATCC 13932)	6.8×10^6
Listeria monocytogenes (ATCC LM 301)	6.2×10^6

Carrier Treatment Controls
Results Expressed as Average colony Forming Units (CFU) per Carrier

Replicate	CFU/Carrier	Average CFU/Carrier
1	1.8×10^4	1.8×10^4
2	2.0×10^4	
3	1.6×10^4	

LMP 102 Lot Number	Replicate	Test Results (Avg. CFU/carrier)	Percent Reduction	Average Percent Reduction
0107F040117	1	6.5×10^1	99.64	99.46
	2	1.7×10^2	99.06	
	3	5.4×10^1	99.70	
0107H150101	1	1.1×10^2	99.39	99.46
	2	6.1×10^1	99.66	
	3	1.2×10^2	99.33	
0107H150202	1	1.8×10^2	99.00	99.41
	2	1.0×10^2	99.44	
	3	3.7×10^1	99.79	

VI CONCLUSION

1. The protocol did not receive prior Agency approval before testing/data collection was initiated. The performance standard proposed (i.e. the test agent meets effectiveness requirements if the test results exhibit a bacterial reduction of at least 99% (2 logs) over the PBS-treated control within five minutes) does not represent the Agency's standard for non-food contact sanitizers. The intended use of the product is novel territory for the Agency, and requires additional label clarification.

VII RECOMMENDATION

1. The Agency cannot extend non-food contact sanitizer registration to the product, LMP-102. As the product is a supplement to EPA approved sanitizers, the label language must reflect this use pattern. The registrant should provide a label explicitly reflecting supplemental use pattern for Agency approval.